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(54) Title: ANTIBODIES TO NON-FUNCTIONAL P<sub>2</sub>X<sub>7</sub> RECEPTOR DIAGNOSIS AND TREATMENT OF CANCERS AND OTHER CONDITIONS

(57) Abstract: The invention concerns a wide range of diseases and conditions, including cancers. The invention provides a probe for detection of such a disease or condition. The probe is able to distinguish between functional P<sub>2</sub>X<sub>7</sub> receptors and non-functional P<sub>2</sub>X<sub>7</sub> receptors. The probe can do this in various ways, one of which is detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors. The invention also provides a method for detecting the disease or condition, using the probe. The invention extends to treatment of the disease or condition, using an antibody, or an epitope capable of generating the antibody, which can distinguish between functional and non-functional P<sub>2</sub>X<sub>7</sub> receptors and bind to the non-functional receptors. Methods of treatment, pharmaceutical compositions and use of the probe and antibody are also included.

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## DIAGNOSIS AND TREATMENT OF CANCERS AND OTHER CONDITIONS

### TECHNICAL FIELD

This invention concerns diagnosis and treatment of diseases, including cancers.

The types of diseases with which this invention is concerned include cancers

- 5 derived from epithelial cells and malignant lymphoma. The invention also concerns other conditions, such as preneoplastic states, irritable bowel syndrome and viral and other infections. It is quite possible that the invention is also applicable to other diseases and conditions.

### BACKGROUND

- 10 Adenosine triphosphate (ATP) can activate ligand-gated purinergic receptors known as P2X receptors. Receptor subtypes P2X<sub>1</sub> to P2X<sub>7</sub> have been identified. It is known that different P2X receptor subtypes are present in many cells, including epithelial cells and leukocytes, including lymphocytes, thymocytes, macrophages and dendritic cells.

- 15 P2X receptors are permeable to calcium ions as well as some other cations, such as potassium and sodium. An influx of calcium ions into a cell via a P2X receptor can be associated with cell death.

- It is believed that the P2X<sub>7</sub> subtype is involved in apoptosis, or programmed cell death, in many cell types. In the presence of ATP, the P2X<sub>7</sub> receptor expressed on  
20 the surface of a cell is capable, within a second, of opening calcium channels through the cell membrane. Continued exposure to ATP can lead to the formation of large pores, within a few seconds to tens of seconds, that enable the cell to be flooded with excess calcium, inducing apoptosis.

- Exposure to ATP does not generally result in apoptosis in the case of epithelial  
25 cancer cells, for example. It has been found that such cells express P2X<sub>7</sub> receptors that are unable to form pores. These are regarded as non-functional receptors.

In human cancer cell lines, such as prostate PC3 and breast MCF7, as well as in animal cell lines including rodent hybridomas, the P2X<sub>7</sub> receptor is found on the cell surface in a non-functional conformation.

The B-cells of patients with malignant lymphoma express non-functional P2X<sub>7</sub> receptors. Lymphoma develops from malignant clones that escape cytolytic destruction. This process leads to the progressive accumulation of malignant B-lymphocytes and thus lymphadenopathy and/or splenomegaly.

### SUMMARY OF THE INVENTION

In a first aspect, this invention provides a probe for detection of a disease or condition, the probe being adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors. Preferably, the probe distinguishes between functional and non-functional P2X<sub>7</sub> receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors or by detecting change in binding of one or more proteins necessary for pore formation in P2X<sub>7</sub> receptors. In an alternate embodiment, the probe detects one or more parts of the P2X<sub>7</sub> receptor exposed in the absence of bound ATP. Such receptor part may include a P2X<sub>7</sub> monomer.

The invention also provides a method for detecting a disease or condition, the method including the steps of using the probe of the invention to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors, providing a receptor expression profile, and comparing the receptor expression profile with that of a normal profile. The change may be detected, for example, as indicated above in connection with the probe itself.

The probe may be natural or artificial. Preferably, the probe is an antibody, which may be polyclonal, monoclonal, recombinant, a humanised antibody or an appropriate fragment thereof. The antibody is preferably directed against an epitope located in an extracellular domain adjacent to a site for binding ATP. In the

case of human P2X<sub>7</sub> receptors, the probe is preferably adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation.

- 5 The probe may be prepared using any suitable technique, as will be readily apparent to one skilled in the art.

It is within the scope of the invention that the probe may distinguish between functional and non-functional receptors through detection of other conformational changes occurring at a site for binding ATP. For example, the change detected may  
10 be in an amino acid other than the proline referred to above. An example of such an amino acid is Pro199. As another example, the change detected may be in some other respect.

The probe may also be adapted to detect other regions of the P2X<sub>7</sub> receptor unchanged by functional state. The conformation of the monomeric subunits  
15 lacking bound ATP may be detectable using the probe, as the epitope chosen may specifically detect the shape of a region of the surface of the receptor accessible only when ATP is not bound. The probe may detect change in binding of one or more proteins, such as accessory or other proteins, necessary for pore formation. Non-limiting examples of such proteins are laminin, integrin, beta-actin, alpha-  
20 actinin and supervillin.

In the present invention, a P2X<sub>7</sub> subtype-specific antibody can be used to specifically detect or bind to non-functional P2X<sub>7</sub> receptors expressed in or on cells forming part of preneoplastic tissue, very early neoplastic tissue, advanced neoplastic tissue and on any neoplastic cell expressing non-functional P2X<sub>7</sub>  
25 receptors. Thus, the P2X<sub>7</sub> receptor is detected or bound only when in the closed or non-functional conformation, even though it may be normally expressed in the cell membranes and may otherwise be partially able to function as a channel.

Further, the conformation of the monomeric subunits lacking bound ATP is also detectable with the antibody, because the epitope chosen specifically detects the shape of a region of the surface accessible only when ATP is not bound.

In the present invention, the non-functional P2X<sub>7</sub> receptors can be detected or  
5 bound by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors, so that the cause of the conformational change in the receptors relates to the interaction of the receptors  
10 with ATP. As set out above, the ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the inflow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors, for reasons that may include a deficit in the local availability of ATP through  
15 production deficit or increase in the rate of degradation. If ATP binding to the receptors is disrupted, the receptor conformation is altered. This can be detected by using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

In the case of human P2X<sub>7</sub> receptors, the specific sequence involved in the  
20 conformational change may include Pro210, which undergoes a change in conformation from the trans form to the cis form in the absence of bound ATP. Thus, in the case of human receptors, an appropriate epitope sequence against which an antibody must be raised may include Pro210, and may extend either side of this residue, to an appropriate extent necessary to induce an antibody response.  
25 By way of non-limiting example, this may include a segment extending from Gly200 to Thr215. Further, a homologous segment from other mammals, such as rat, may be used where this cross-reacts with human tissue. As an example, the same segment Gly200 to Thr215 in rat may be used, although there are two amino acid substitutions in the rat sequence compared with the human sequence.

In the case of non-human receptors, the specific sequence may be ascertained by suitable experiment.

The detection of non-functional P2X<sub>7</sub> receptors according to the invention may show a distribution pattern in which functional receptors (and hence normal cells) may remain essentially unlabelled. However, non-functional conformations of P2X<sub>7</sub> receptors may be detected, initially in the nuclei and cytoplasm of cells, at a very early stage in preneoplasia. For example, in the case of epithelial cell cancer, using the method of the invention it may be possible to detect preneoplasia several years prior to the normal pathological appearance of cancer as detected by haematoxylin and eosin ("H & E") stained slides of biopsied tissues. Thus, cancers such as prostate, skin and breast may be detected far earlier than is currently the case, with the advantages of introduction of early therapy.

The full scope of the diseases and conditions which may be detected by the probe and method of the invention has not yet been ascertained. However, it is believed that these include epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as blood cancers including malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as Mycobacterium tuberculosis. Infection may cause non-functional receptors to be expressed either directly through inhibition of co-factors required for functionality, or through the up-regulation of co-factors acting to inhibit P2X<sub>7</sub> function on epithelial or other cells, so rendering the infected cell less amenable to destruction by apoptosis.

Unless otherwise indicated, the term "disease or condition" as used herein is intended to include all those specific diseases and conditions set out in the preceding paragraph.

In the specific case of irritable bowel syndromes ("IBS"), it has now been found that, in patients with this condition, the gut mucosa, that normally expresses P2X<sub>7</sub> receptors in the widely distributed lymphocytes present in the stroma beneath the

epithelium, becomes up-regulated. In affected patients, this increased expression can be observed from duodenum to rectal mucosa. The increased expression may be found in isolated regions, or to be generally increased over the entire length of the intestinal tract in more extreme cases.

- 5 In the least affected cases, total P2X<sub>7</sub> receptors are up-regulated, but these are all functional and they do not penetrate into the epithelium. In more severe cases, total P2X<sub>7</sub> receptor expression is even higher, and the most affected areas of the gut exhibit receptors that are non-functional. These may be localised to caecal mucosa, for example, and may penetrate into the epithelium. The most severe cases are  
10 those in which total P2X<sub>7</sub> receptor expression is further increased and most of the receptors are non-functional with increased epithelial cell penetration.

As already discussed, non-functionality of P2X<sub>7</sub> receptors is caused by lack of appropriate binding of the ATP agonist to the receptors. The reasons for this may include a deficit in the local availability of ATP through production deficit or  
15 increase in rate of degradation through ecto-ATPase enzymatic degradation of ATP. If ATP binding to the receptors is disrupted, the receptor conformation is altered as already discussed, and this can be detected using the probe of the invention. However, the detection of total P2X<sub>7</sub> receptor distribution is best achieved using an epitope to other regions of the extracellular domain of the P2X<sub>7</sub>  
20 receptor that is not affected by ATP binding.

It is within the scope of this invention to use one or two P2X<sub>7</sub> subtype-specific antibodies to specifically distinguish between total P2X<sub>7</sub> distribution and the proportion of receptors that are non-functional and expressed in gut mucosa. Thus the two antibodies used together can detect both total receptor count and those  
25 receptor channels present only in a close-gated or non-functional conformation. The first antibody is adapted to detect total P2X<sub>7</sub> receptor expression. The probe comprising or attached to the antibody of the invention can provide the second antibody for detection of IBS, not only distinguishing between functional and non-

functional P2X<sub>7</sub> receptors, but also allowing for detection of other regions in which the receptor is unchanged by functional state. The antibodies may be used separately or together. Preferably, they are used in combination.

The detection of all P2X<sub>7</sub> receptors, separately from non-functional P2X<sub>7</sub> receptors,  
5 determines the severity of the condition. Expression of non-functional P2X<sub>7</sub> receptors in the gastrointestinal mucosa occurs in a pattern in which normal cells remain essentially unlabelled. Thereafter, the non-functional conformation of P2X<sub>7</sub> is first detected in the stroma underneath the epithelium ranging from isolated patches in mild cases of the syndrome to extensive expression throughout the  
10 length of the gastrointestinal tract with isolated patches of infiltration of non-functional receptors into the epithelium.

The invention also provides a method of diagnosing irritable bowel syndrome, comprising detecting the P2X<sub>7</sub> expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells  
15 and/or tissue. Preferably, the detection of the P2X<sub>7</sub> expression profile includes use of one or more antibodies. Further, it is preferred that such antibody or antibodies are different from the probe of the invention in that they do not detect change in relation to binding of ATP to the P2X<sub>7</sub> receptors. The preparation of such antibodies will be readily apparent to one skilled in the art.

20 The invention also includes use of one or more antibodies to diagnose irritable bowel syndrome.

The diagnostic can be used in standard microscopy employing standard immunohistochemical techniques.

Therapeutic treatment for this condition is discussed below, in connection with the  
25 third aspect of this invention.

Diagnosis using the probe and method of the invention may be carried out using in situ imaging techniques to detect distribution in body tissues. In addition, standard



microscopy, confocal microscopy and fluorescence activated cell sorting may be used. Normal immunohistochemical techniques for testing lymph, prostate, breast, skin, lung, uterus, bladder, cervix, stomach, oesophagus and similar biopsies, also fine needle aspirates of breast and other tissue and cell smears such as those taken  
5 for the detection of cervical cancer, may be used. Other techniques may be used with the probe and method of the invention.

This invention provides an antibody for treating a disease or condition, the antibody being adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors and being adapted to bind only to non-functional  
10 receptors. Preferably, the antibody distinguishes between the functional and non-functional receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors, or by detecting change in binding of one or more proteins necessary for pore formation in P2X<sub>7</sub> receptors and being adapted to bind only to non-functional receptors. In another embodiment, the antibody  
15 distinguishes between the functional and non-functional receptors by detecting parts of the receptor exposed in the absence of bound ATP.

The antibody for treating diseases and conditions may be the same as the antibody which may be used as the probe for diagnosing diseases and conditions. Such an antibody could be used to treat skin cancers topically, for example. For systemic  
20 treatment of cancer, the antibody or its active fragments should be humanised in order to minimise undesirable immune response side effects.

The antibody of the invention may be used to treat diseases or conditions in mammals, including humans. Examples of the diseases or conditions have been set out above in connection with the probe of the invention.

25 The invention also provides an epitope capable of causing the generation of the antibody of the second aspect of the invention. The epitope preferably includes Pro210 and encompasses the segment Gly200 to Thr215 (in the P2X<sub>7</sub> sequence of the human receptor). The epitope should preferably have attached to the C-terminal

end a Cys residue that is cross-linked to diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS), so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.

This specific peptide conformation is intended to be presented to humans or  
5 animals with one or more diseases or conditions, especially epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as *Mycobacterium tuberculosis*. The patient will preferably mount an immune  
10 response to the applied conjugated epitope and so generate antibodies recognising the non-functional P2X<sub>7</sub> receptors present on the surface of the affected cells, thus binding to them and alerting the appropriate immune cell to destroy the complexed cells. Other cells primed for cell death may also be affected.

It is to be understood that the sequence referred to above is not limiting on the  
15 scope of the invention, which includes alternate sequences and carriers and cross-linkers that similarly produce a specific immune response, preferably against only non-functional P2X<sub>7</sub> receptors, preferably ignoring all functional receptors expressed on cell surfaces, and so avoiding side effects.

The invention, in this second aspect, also provides for the use of the antibody of the  
20 invention as a therapeutic vehicle for treatment of a disease or condition in a patient to regulate programmed cell death by targeting aberrant or non-functional P2X<sub>7</sub> receptors expressed on the surface of cells, while leaving all cells expressing normal (functional) receptors untouched. The invention also covers the use of the epitope of the invention to cause the generation of the antibody, as above.

25 The invention also provides a pharmaceutical composition for treatment or prevention of a disease or condition in a patient, the composition including a pharmaceutically effective amount of an antibody, or an epitope to cause the

generation of such an amount, capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional P2X<sub>7</sub> receptors.

The pharmaceutically effective amount of the antibody or epitope will vary according to the patient and the nature of the disease or condition. These variables  
5 can be ascertained by one skilled in the art.

The pharmaceutical composition of the invention may be administered in conjunction with a pharmaceutically acceptable carrier, which may be any of those known in the art or devised hereafter and suitable for the intended use. As well as carriers, the pharmaceutical compositions of the invention may include other  
10 ingredients, including dyes, preservatives, buffers and antioxidants, for example.

The pharmaceutical composition of the invention may take any desired form and may be administered, for example, in the form of an ointment, cream, solution, suspension, powder, tablet, capsule, suppository or pessary.

The pharmaceutical composition of the invention may be administered in any  
15 suitable way, which may include oral, parenteral, intravenous, intramuscular, subcutaneous or topical administration.

The invention also provides a method of treating or preventing a disease or condition in a patient, the method including administering to the patient a pharmaceutical composition according to the invention.

20 The invention also provides the use of the pharmaceutical composition of the invention, in the treatment or prevention of a disease or condition, in a patient.

It will be apparent to one skilled in the art that the pattern of use of the pharmaceutical composition of the invention may need to be altered for optimum effect. It may be necessary to take into account the nature of the disease or  
25 condition as well as its severity.

The third aspect of the invention focuses on the expression of ATPases (enzymes) that control the supply of ATP to P2X<sub>7</sub> receptors, for example in the B-cells of a patient having malignant lymphoma. Channel opening of P2X<sub>7</sub> receptors on leukocytes is terminated through the rapid hydrolysis of ATP agonist by ecto-ATPases and ecto-ATP diphosphohydrolases (ecto-ATPDases). These enzymes regulate numerous physiological processes that are dependent on ATP. Substrate specificity of ATPase and ATPDase activity on lymphocytes indicates the presence on the lymphocytes of more than one type on the cell surface, including CD39. Proliferation of one or more of these ATPases or ATPDases could limit the supply of ATP needed to control P2X<sub>7</sub> pore formation and the subsequent programmed cell death needed to regulate B-cell numbers.

Similarly, it is believed that, in the case of IBS, proliferation of ATPases may contribute to lack of appropriate binding of the agonist ATP to the P2X<sub>7</sub> receptors.

Accordingly, in this third aspect, the invention provides a preparation for treatment or prevention of a disease or condition in a patient, the preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X<sub>7</sub> receptors in the patient's cells or tissues. The invention also provides a method of treating or preventing a disease or condition in a patient, the method including the step of administering to the patient a preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X<sub>7</sub> receptors in the cells or tissue of the patient.

Examples of such ATPases may be CD39 or CD73.

Such a substance may take the form of an ATP analogue, preferably non-hydrolysable, and specific for P2X<sub>7</sub>, or another substance that inhibits the action of local ATPases depleting the availability of ATP for the P2X<sub>7</sub> binding site. The preparation may be in the form of a humanised antibody directed specifically against non-functional P2X<sub>7</sub> receptors.

The disease or condition is preferably malignant lymphoma or IBS but the invention may also extend to other diseases or conditions, including other epithelial cell or blood cancers or viral and other pathological infections.

In the case of malignant lymphoma, the ATPases control the local supply of ATP  
5 to the P2X<sub>7</sub> receptors so as to reduce the concentration of ATP available for binding to the P2X<sub>7</sub> receptors and so deactivate them leading to a significant reduction in programmed B-cell death. These ATPases may be specifically expressed on the surface of the B-cells and appear to be up-regulated in malignant lymphoma. Preferably, application of a specific ATPase inhibitor may be used to  
10 regulate the availability of ATP on the P2X<sub>7</sub> receptors, so regulating programmed B-cell death.

For treatment of malignant lymphoma, the substance may include a synthetic agonist capable of blocking ATPases or ATPDases, of the form of non-hydrolysable P2X<sub>7</sub> agonist.

15 In relation to irritable bowel syndrome, administration of the preparation of the invention is intended to restore receptor function that may be depleted through overactivity of the muscle underlying the affected region of mucosa. The preparation of the invention may act on the mucosa directly to remove these non-functional receptors and thereby restore local normal gastrointestinal secretory  
20 mechanisms. Therapeutic treatment is aimed at restoring the local supply of ATP to the non-functional receptors, so that normal receptor function is restored. The consequences of control of receptor function include restoration of normal control of gastrointestinal secretions and peristalsis. This may be achieved by application of enteral or systemic supply of synthetic P2X<sub>7</sub>-specific agonist, preferably non-  
25 hydrolysable by ATPases, by systemic application of an antibody directed against non-functional P2X<sub>7</sub> receptors, preferably a small humanised specific antibody to remove the non-functional receptors, leaving only functional receptors.

If abnormalities of peristalsis in the underlying smooth muscle are responsible for depleting the local availability of ATP for binding to the normal P2X<sub>7</sub> receptors, treatment may involve restoration of this natural supply of agonist by means of a limit on the uptake or use of ATP by the smooth muscle through application of a treatment to temporarily limit gut motility.

The invention also provides a pharmaceutical composition for treatment of a disease or condition, the composition including a pharmaceutically effective amount of one or more substances adapted to regulate the expression of ATPases (enzymes) that control the supply of ATP to P2X<sub>7</sub> receptors.

10 The invention in all its aspects extends to such similar applications that could be made in other medical conditions in which aberrant P2X<sub>7</sub> receptors are involved as a result of viral infection where the virus is protected in the infected cell by up-regulating non-functional P2X<sub>7</sub> receptor or where such receptors are up-regulated from the normal cell condition.

15 The invention also provides a method of treating irritable bowel syndrome, comprising administering to a patient a pharmaceutical composition as defined above.

The invention also provides the use of such a pharmaceutical composition in the treatment of irritable bowel syndrome.

20 The pattern of use of one or more of the above pharmaceutically effective agents may need to be altered for optimum effect.

Expressed another way, the invention provides a method of treating irritable bowel syndrome, the method including administering a composition adapted to restore P2X<sub>7</sub> receptor function. The receptor function may have been depleted through overactivity of the muscle underlying the affected region of mucosa. The composition may be the same as that set out above for the substance included in the preparation of the invention.

In a further aspect, the invention provides a method for distinguishing between different conformations of proteins by using an epitope capable of causing the generation of an antibody, or the antibody itself, to effect specific pharmaceutical outcomes (active as well as passive immunisation) from binding to all members of  
5 the proteins with a selected conformation. An example of this would be prion proteins in the conformation that leads to the condition vCJD. The abnormal form of the protein could be targeted by a specific antibody or epitope causing the generation of the antibody, preferably humanised and reduced in size for optimum pharmacological effect.

10

## DESCRIPTION OF THE INVENTION

### DIAGNOSTIC APPLICATIONS

Descriptions are provided here by way of example, using the specific non-functional P2X<sub>7</sub> antibody in animals and demonstrating the universal application of  
15 the probe and method of the invention to the diagnosis of most cancers in humans and other mammals.

In prostate tissue from humans and mammals, such as cats and dogs, when the antibody of the invention is used for diagnosis, no labelling is obtained in the absence of cancer or pre-cancerous lesions. However, the diagnostic method of the  
20 invention reveals first signs of neoplastic change while there is still no accompanying morphological changes detectable by H&E stain.

At this stage, it is necessary to stain for the receptor units first appearing in the nuclei of epithelial cells. These migrate to the cytoplasm in later stages of the disease, acting as a field effect throughout the prostate, so that less tissue need be  
25 biopsied to be certain of the existence of a tumour. In later stages of the disease, the staining becomes more confined to the apical epithelium.

Similarly, other epithelial cell cancers, like breast, lung, colon and skin in humans and in other mammals, such as cats and dogs, can be detected with margins as there is no longer a clear field effect in these other tissues.

The same stage development is seen in these other tissues, like breast and cervix,  
5 with nuclear stain preceding cytoplasmic stain, while normal tissue is unstained. Affected ducts and lobules in breast tissue are readily detected due to the local field effect within the individual affected duct system in the breast even where normal morphology suggests there is no cancer. Adjacent unaffected ducts appear unstained. Similarly, affected lymph nodes, directly draining tissue containing a  
10 tumour, show signs of the tumour through the field effect of affected lymphocytes. Thus, sentinel nodes can be detected without there being any metastatic cellular spread to the node.

Skin cancers, such as basal cell carcinoma and squamous cell carcinoma as well as malignant melanomas show positive staining for non-functional receptors and  
15 channel components (monomers) in keratinocyte and melanocyte layers with clear margins beyond which normal skin is unlabelled on both epidermis and deep within the dermis.

All tested mammalian cancer cell lines such as human prostate (PC3) and breast (MCF7) and rodent hybridomas are positive for the non-functional receptors on the  
20 cell surface so that apoptosis is inhibited in these cancer cells. The general application of this diagnostic is seen by way of the same label on mouse hybridoma cells showing the ubiquitous nature of the receptor in other animal types besides human. Normal human B-cell lymphocytes show that functional P2X<sub>7</sub> receptors are expressed on the cell surface, so enabling apoptosis when necessary, while human  
25 B-cell lymphocytes from patients with malignant lymphoma show that non-functional P2X<sub>7</sub> receptors are expressed on the cell surface, so curtailing apoptosis.

#### THERAPEUTIC APPLICATIONS



Targeting this apparently ubiquitous P2X<sub>7</sub> non-functional conformer expressed on the cell surface of cancer cells attempting to undergo apoptosis may be used to treat most cancers in humans and other mammals. Examples are set out below:

#### EXAMPLE 1

- 5 Mouse hybridoma cells were grown on a macrophage base both in the presence and absence of affinity purified antibody to non-functional P2X<sub>7</sub>. Cell counts revealed that over 4 days while cells grew in the presence only of purified normal IgG from 1 to  $7 \times 10^4$ , the presence of non-functional P2X<sub>7</sub> antibody kept the cell count to only  $1.5 \times 10^4$ .
- 10 The hybridoma cells were injected into mice together with normal IgG as a control or with affinity purified antibody to non-functional P2X<sub>7</sub>.  $10^6$  hybridoma cells were preincubated for 6 hours with either an antibody raised against non-functional (ATP-depleted) P2X<sub>7</sub> (protein G purified but not affinity purified) or IgG at the same concentration in a volume of 0.5 mL that was then injected into groups of
- 15 mice. A second identical injection was performed on Day 2.

Tumour weight was measured on Day 11. Mean tumour weight in the control group of 10 mice, of whom 9 developed tumours, was 4.0 g, while in the group of 10 mice exposed to the P2X<sub>7</sub> antibody, of whom 9 also developed tumours, the mean weight of tumour was only 1.1 g.

#### 20 Example 2

- Specific affinity purified antibody (to greatly improve specificity) was applied to 3 human basal cell carcinomas ("BCC") either as a liquid held in place for 7 days or suspended in a dimethicone cream base. No trace of the BCC lesions was detectable after treatment, while control skin was entirely unaffected due to the
- 25 absence of the protein target.

It is believed that application to patients in general would involve production of a humanised monoclonal antibody (such as herceptin) so that internal cancers could be treated with the same efficacy as is revealed with topical application. All normal functional P2X<sub>7</sub> expressed on the cell surfaces of cells such as lymphocytes would  
5 need to remain unaffected by the presence of the antibody to avoid side effects.

The antibody should therefore only bind to proteins expressed on the cell surface of cells attempting to but unable to initiate apoptosis. Thus all cells targeted would be only those attempting to kill themselves through programmed cell death, including cancer cells. The P2X<sub>7</sub> receptors on these cells, particularly cancer cells, would be  
10 in a non-functional or ATP-depleted state.

#### ACTIVE IMMUNISATION

Active immunisation may also be used for therapeutic purposes. In this case the humans or other mammals need to be immunised against a specific epitope or epitopes that are in a conformation that mimics the conformation adopted only by  
15 the receptors in their non-functional (ATP-depleted) shape on the cell surface.

Conformational flexibility that includes partial exposure of an epitope shape that is present in functional receptors should be avoided. The cis configuration of the epitope Gly200-Thr215 as an example should be fixed before use by appropriate means. As added proof that this concept is sound is the observation that numerous  
20 animals including mice, rabbits and sheep used to raise the antibodies have not been immuno-compromised. None of these many animals have ever developed any tumours.

#### INDUSTRIAL APPLICABILITY

The invention in all its aspects has application to the fields of human and  
25 veterinary medicine and health, with the potential to enable early and accurate diagnosis of diseases and effective treatment, which in many cases is far less invasive or traumatic than those available in the prior art.

**CLAIMS**

1. A probe for detection of a disease or condition, the probe being adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors.
- 5 2. The probe of claim 1, wherein the probe is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.
3. The probe of claim 1, wherein the probe is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting  
10 change in binding of one or more proteins necessary for pore formation in P2X<sub>7</sub> receptors.
4. The probe of claim 3, wherein the probe is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting one or more parts of the receptor exposed in the absence of bound ATP.
- 15 5. The probe of claim 4, wherein the part includes a P2X<sub>7</sub> monomer.
6. The probe of any one of claims 1 to 5 which is natural or artificial.
7. The probe of any one of claims 1 to 5, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell  
20 cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.
8. The probe of claim 7, in which the antibody is directed against an epitope of each receptor located in an extracellular domain adjacent to a site for binding ATP.

9. The probe of any one of claims 1 to 5, wherein the receptors are mammalian P2X<sub>7</sub> receptors and the probe is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation.  
5
10. The probe of any one of claims 1 to 5, wherein the receptors are mammalian P2X<sub>7</sub> receptors and the probe is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 199 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 199 is in the cis conformation.  
10
11. The probe of claim 9, wherein the probe is or includes an antibody raised against an epitope sequence of the P2X<sub>7</sub> receptor extending from Gly200 to Thr215.
12. The probe of any one of claims 1 to 11, wherein the disease or condition is  
15 chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.
13. The probe of claim 12, wherein the virus or organism is HIV or  
20 Mycobacterium tuberculosis.
14. The probe of claim 7, wherein the condition is irritable bowel syndrome and the antibody is also capable of detecting other regions of the P2X<sub>7</sub> receptor unchanged by functional state.
15. The probe of claim 7 or claim 14, wherein the condition is irritable bowel  
25 syndrome and the antibody is used in combination with a second antibody capable of detecting total P2X<sub>7</sub> expression.

16. A method for detecting a disease or condition, the method including the steps of:
- using the probe claimed in any one of claims 1 to 10 to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors,
- 5 providing a receptor expression profile, and
- comparing the receptor expression profile with that of a normal profile.
17. The method of claim 16, wherein the receptor expression profile is that of non-functional receptors.
18. The method of claim 16, wherein the probe is adapted to distinguish between
- 10 functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.
19. The method of claim 16, wherein the probe is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting change in binding of one or more proteins necessary for pore formation in
- 15 P2X<sub>7</sub> receptors.
20. The method of claim 16, wherein the probe is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting one or more parts of the receptor exposed in the absence of bound ATP.
21. The method of claim 16, wherein the part includes a P2X<sub>7</sub> monomer.
- 20 22. The method of any one of claims 16 to 21, wherein the receptor expression profile is provided using in situ imaging techniques.
23. The method of any one of claims 16 to 21, wherein the receptor expression profile is provided using microscopy, confocal microscopy or fluorescence activated cell sorting.

24. Use of the probe claimed in any one of claims 1 to 15 to detect a disease or condition.
25. An isolated cell or tissue sample complexed with a probe as claimed in any one of claims 1 to 15.
- 5 26. A method of diagnosing irritable bowel syndrome, comprising detecting the P2X<sub>7</sub> expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue.
27. The method of claim 26, wherein the detection of the P2X<sub>7</sub> expression profile includes use of one or more antibodies.
- 10 28. Use of one or more antibodies to diagnose irritable bowel syndrome.
29. A method of treating irritable bowel syndrome, the method including administering a composition adapted to restore P2X<sub>7</sub> receptor function.
30. An antibody for treating or preventing a disease or condition, the antibody being adapted to distinguish between functional P2X<sub>7</sub> receptors and non-  
15 functional P2X<sub>7</sub> receptors and to bind only to non-functional receptors.
31. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.
- 20 32. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by detecting change in binding of one or more proteins necessary for pore formation in P2X<sub>7</sub> receptors.
- 25 33. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional P2X<sub>7</sub> receptors and non-functional P2X<sub>7</sub> receptors by

detecting one or more parts of the receptor exposed in the absence of bound ATP.

34. The antibody of claim 30, wherein the part includes a P2X<sub>7</sub> monomer.
35. The antibody of any one of claims 30 to 34 which is polyclonal, monoclonal,  
5 recombinant, a humanised antibody or an appropriate fragment thereof.
36. The antibody of any one of claims 30 to 34, wherein the receptors are mammalian P2X<sub>7</sub> receptors and the antibody is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in  
10 which the proline at amino acid 210 is in the cis conformation.
37. The antibody of claim 36, which is raised against an epitope sequence of the P2X<sub>7</sub> receptor extending from Gly200 to Thr215.
38. The antibody of any one of claims 30 to 34, wherein the receptors are mammalian P2X<sub>7</sub> receptors and the antibody is adapted to distinguish between  
15 functional receptors having a sequence in which proline at amino acid 199 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 199 is in the cis conformation
39. The antibody of any one of claims 30 to 37, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus,  
20 stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.
40. The antibody of claim 39, wherein the virus or organism is HIV or Mycobacterium tuberculosis.
- 25 41. An epitope adapted to cause the generation of the antibody of any one of claims 30 to 40.

42. The epitope of claim 41 which has attached to the C-terminal end a Cys residue that is cross-linked to diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS), so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.
43. Use of the antibody of any one of claims 30 to 40 as a therapeutic vehicle for treatment of a disease or condition in a patient to regulate programmed cell death by targeting aberrant or non-functional P2X<sub>7</sub> receptors expressed on the surface of cells, while leaving all cells expressing normal (functional) receptors untouched.
44. Use of the epitope of claim 41 or 42 to cause the generation of the antibody of any one of claims 30 to 40.
45. A pharmaceutical composition for treatment or prevention of a disease or condition in a patient, the composition including a pharmaceutically effective amount of an antibody as claimed in any one of claims 30 to 40, or an epitope to cause the generation of such an amount, capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional P2X<sub>7</sub> receptors.
46. A preparation for treatment or prevention of a disease or condition in a patient, the preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X<sub>7</sub> receptors in the patient's cells or tissues.
47. A method of treating or preventing a disease or condition in a patient, the method including the step of administering to the patient a preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X<sub>7</sub> receptors in the cells or tissue of the patient.

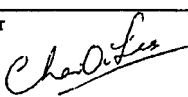


48. The preparation of claim 46, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and  
5 infection by a virus or other pathological organism.
49. The method of claim 47, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and  
10 infection by a virus or other pathological organism.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00061

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int. Cl. <sup>7</sup> : C07K14/705,16/28; A61K 39/395; A61P 35/00, 1/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CA, MEDLINE, BIOSIS, WPIDS, BIOTECHABS (KEYWORDS: P2X <sub>7</sub> , ANTIBODY, IMMUNOGLOBIN, CONFORMATION, ATP ANALOGUE, ATPASE)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Groschel-Stewart U et al, "Localisation of P2X <sub>5</sub> and P2X <sub>7</sub> receptors by immunohistochemistry in rat stratified squamous epithelia", Cell Tissue Research 1999 vol 296 (3), pages 599-605 See whole document especially page 604 2 <sup>nd</sup> col last para.	1-49
X	Buell G et al, "Blockade of human P2X <sub>7</sub> receptor function with a monoclonal antibody", Blood vol 92 No 10, 1998, pages 3521-3528 See whole document especially page 3526 col 2 last para to page 3527 col 2 last para.	1-8, 12-25, 39, 41-45
X	Wiley et al, "A single nucleotide polymorphism is associated with loss of function of the monocyte P2X <sub>7</sub> receptor", Blood vol 96 (11 pt 1) Nov 2000 page 17a (Abstract)	1-8, 12, 16-25, 30-35, 39, 41, 44, 45
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 2 April 2002		Date of mailing of the international search report 9 APR 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		Authorized officer  O.L. CHAI Telephone No : (02) 6283 2482

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00061

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Peng L et al, "P2Z purinoceptor, a special receptor for apoptosis induced by ATP in human leukemic lymphocytes", Chinese Medical Journal 1999, 112 (4) pages 356-362 See whole document especially page 361 col 1 para 3.	46-49
P, X	Chesswill IP et al, "Dynamics of P2X <sub>7</sub> receptor pore dilation: pharmacological and functional consequences", Drug Development Research 2001 vol 53 (2/3) pages 60-65 See whole document especially page 63 col 1 last para to page 64 col 1 2 <sup>nd</sup> para.	1-49
P, X	Wiley JS et al, "Genetic polymorphisms of the human P2X <sub>7</sub> receptor and relationship to function", Drug Development Research 2001 vol 53 (2/3) pages 72-76 See whole document especially page 74 col 1 para 2 to page 75 col 1 para 1.	1-49
A	Virgilio F et al, "Purinergic P2X <sub>7</sub> receptor: a pivotal role in inflammation and immunomodulation", Drug Development Research 1998 vol 45 (3/4) pages 207-213 See whole document especially page 209 col 1 para 3 to page 210 col 1 para 1.	
A	Ferrari D et al, "ATP-mediated cytotoxicity in microglial cells", Neuropharmacology 1997 vol 36 (9) pages 1295-1301 See whole document.	
A	US 6133434 (GLAXO) 17 October 2000 See whole document.	
P, A	Gu B et al, "A Glu-496 to Ala Polymorphism leads to loss of function of the human P2X <sub>7</sub> receptor", J. Biol. Chem. 2001 vol 276 (14) pages 11135-11142 See whole document.	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00061

**Box I** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos : 1-8, 12-35, 39-44, 46-49  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Claims 1-8, 12-35, 39-44  
It is known that the P<sub>2</sub>X<sub>7</sub> receptors are present in many cell types and that the non-functionality of the receptors is caused by lack of appropriate binding of the ATP agonist to the receptor (see specification (continue in supplemental box))
3. ☐ Claims Nos :  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

There are two different groups of inventions as follows:

Group 1. Claims 1-45 are directed to a probe that distinguish between functional P<sub>2</sub>X<sub>7</sub> receptors and non-functional P<sub>2</sub>X<sub>7</sub> receptors, method of using the probe in diagnostic and treatment of disease and pharmaceutical composition.

(continue in supplement box)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00061

**Supplemental Box**

(To be used when the space in any of Boxes I to VIII is not sufficient)

**Continuation of Box No: I**

page 2 paragraphs 1-2). These claims are mere desiderata and do not contain any technical features to allow for a meaningful search.

Claims 46-49

It is known that  $P_2X_7$  receptors are activated by ATP. A preparation that regulate ATPases to control the supply of ATP to  $P_2X_7$  could include any ATP analogue. No meaningful search can be carried out based on the claims as drafted.

Nonetheless, two searches have been carried out in an attempt to cover the claims.

**Continuation of Box No: II**

Group 2. Claims 46-49 are directed to a preparation and method for treatment or prevention of disease that uses one or more substances to regulate the expression of ATPases that control the supply of ATP to  $P_2X_7$  receptors.

These groups of invention are not so linked to form a single general inventive concept. There are no special technical features that are common to these two groups of claims. Thus the international application does not relate to one invention only or to a single general inventive concept, a priori.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/AU02/00061**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
US 6133434	NONE

END OF ANNEX